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Review

Always cleave up your mess: targeting collagen degradation to treat tissue fibrosis

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McKleroy W, Lee TH, Atabai K. Always cleave up your mess: targeting collagen degradation to treat tissue fibrosis. *Am J Physiol Lung Cell Mol Physiol* 304: L709–L721, 2013. First published April 5, 2013; doi:10.1152/ajplung.00418.2012.—Pulmonary fibrosis is a vexing clinical problem with no proven therapeutic options. In the normal lung there is continuous collagen synthesis and collagen degradation, and these two processes are precisely balanced to maintain normal tissue architecture. With lung injury there is an increase in the rate of both collagen production and collagen degradation. The increase in collagen degradation is critical in preventing the formation of permanent scar tissue each time the lung is exposed to injury. In pulmonary fibrosis, collagen degradation does not keep pace with collagen production, resulting in extracellular accumulation of fibrillar collagen. Collagen degradation occurs through both extracellular and intracellular pathways. The extracellular pathway involves cleavage of collagen fibrils by proteolytic enzyme including the metalloproteinases. The less-well-described intracellular pathway involves binding and uptake of collagen fragments by fibroblasts and macrophages for lysosomal degradation. The relationship between these two pathways and their relevance to the development of fibrosis is complex. Fibrosis in the lung, liver, and skin has been associated with an impaired degradative environment. Much of the current scientific effort in fibrosis is focused on understanding the pathways that regulate increased collagen production. However, recent reports suggest an important role for collagen turnover and degradation in regulating the severity of tissue fibrosis. The objective of this review is to evaluate the roles of the extracellular and intracellular collagen degradation pathways in the development of fibrosis and to examine whether pulmonary fibrosis can be viewed as a disease of impaired matrix degradation rather than a disease of increased matrix production.

collagen degradation; extracellular matrix; matrix metalloproteinases; pulmonary fibrosis; UIP

PULMONARY FIBROSIS, CHARACTERIZED by replacement of the normal alveolar architecture with collagen-rich matrix (140, 178), is a common pathological response to lung injury. Fibrosis can occur because of aberrant remodeling during recovery from acute lung injury, in the setting of systemic autoimmune and inflammatory disease, or as an idiopathic process without identifiable antecedent cause (48, 59, 100). Once fibrosis is established there are no effective pharmacological interventions that can reverse the process, leaving patients with significant pulmonary compromise and in many cases progressive clinical deterioration ultimately leading to death (138). As with many fundamental biological processes, the production, deposition, and resorption of collagen, the main component of fibrotic scar, are dynamic processes with the balance between collagen production and removal determining tissue architecture (43, 91). For fibrosis to develop there has to be a disruption of this homeostasis favoring matrix production over degradation. From this perspective, the development of pulmonary fibrosis represents a failure of matrix degradation as much as matrix overproduction. The pathogenesis of pulmonary fibrosis is complex and has been recently reviewed (31, 46, 79, 165). The objective of this article is to review our understanding of the pathways that regulate collagen degradation and how they relate to the development and resolution of fibrosis.

The Clinical Impact of Pulmonary Fibrosis

Fibrosis is the final common pathway of a myriad of insults and injuries to the lung (29). Increased production of extracellular matrix (ECM) is a normal response to parenchymal damage that serves to preserve tissue integrity in anticipation of tissue repair and remodeling. Fibrosis occurs when there is a failure of compensatory remodeling and reestablishment of normal tissue architecture. Lung fibrosis can occur after lung infections, as part of the pathological response to cigarette smoke in chronic obstructive pulmonary disease, as a response to drug or radiation treatment, or as one of the interstitial lung diseases (ILD). Although many different etiologies can induce a fibrotic response in the lung, the persistence and progression of fibrosis only occur in certain diseases (Fig. 1). A single episode of lung infection rarely leads to significant fibrosis. In acute bacterial pneumonias, even when severe, residual areas

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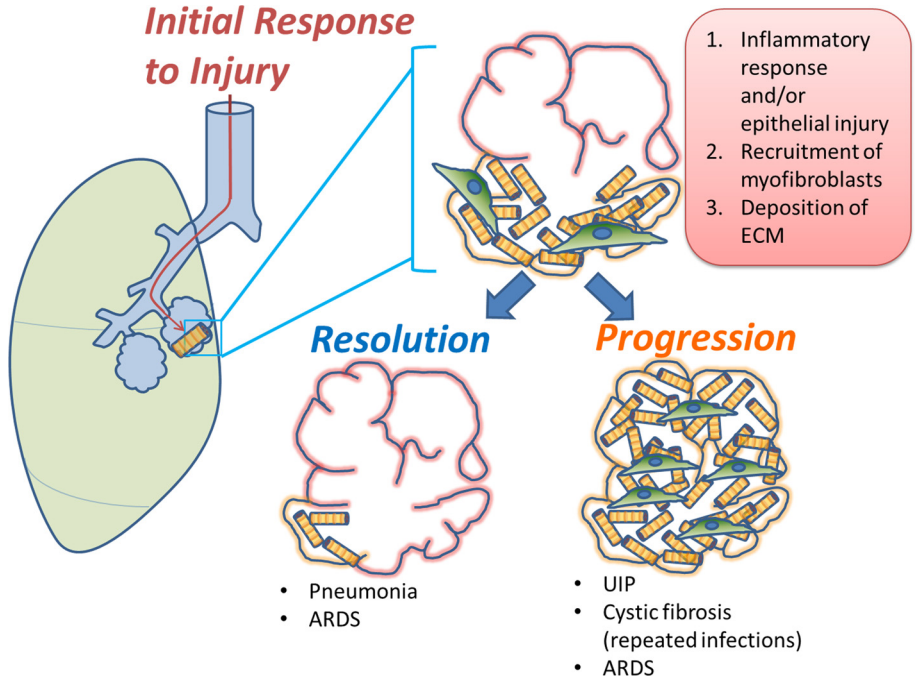

Fig. 1. Many etiologies can induce a fibrotic response in the lung, but fibrosis only persists in certain types of disease. ECM, extracellular matrix; UIP, usual interstitial pneumonitis; ARDS, acute respiratory distress syndrome.

of fibrosis generally do not impact long-term patient quality of life and in many cases are resolved with tissue remodeling leaving near-normal lung architecture. Repeated bouts of infection, however, can lead to a significant fibrotic response as seen in cystic fibrosis (61). A subset of patients with acute respiratory distress syndrome (ARDS) develop a fibroproliferative phase characterized by accumulation of collagen-rich material within the air spaces and severe gas-exchange abnormalities (164). Interestingly, even these patients can have resolution of the fibrotic response over time (114).

Pulmonary fibrosis that occurs in the setting of ILD is a more insidious and problematic process that can be progressive. Of the several disease processes that collectively make up the ILD, usual interstitial pneumonitis (UIP), often referred to as idiopathic pulmonary fibrosis (IPF), carries the worst prognosis (139). Although most of the other disorders respond to varying degrees with treatment of the underlying etiology, UIP is uniformly progressive with a median life expectancy of approximately 3 years after diagnosis. For the purposes of this review, we will use the term IPF when discussing literature that did not use the term UIP in classifying their patient populations.

Production of Excess Collagen

An excess in collagen production is necessary for the development of pulmonary fibrosis. The cells responsible for collagen production are believed to be fibroblasts and myofibroblasts (47, 188). Myofibroblasts are differentiated fibroblasts that express α-smooth muscle actin and secrete significantly greater amounts of collagen and ECM components than their fibroblast counterparts (188). The source of fibroblasts that differentiate into myofibroblasts in fibrotic tissue is an area of contention, with studies supporting migration of local fibroblasts into the alveolar space (134), transformation of local epithelial cells into cells expressing mesenchymal markers through epithelial-to-mesenchymal transformation (EMT) (77), transformation of lung pericytes and pleural mesothelial cells into myofibroblasts (120, 123), and homing of bone marrow-derived cells of the monocyte lineage (fibrocytes) into areas of injury (134). Since local fibroblasts populations are plentiful in the lung it may seem surprising that other cellular sources are required to produce myofibroblasts. Although the exact importance of different populations has yet to be elucidated, there is some evidence to suggest that the source of cells that differentiate into myofibroblasts has pathological relevance. For example, neutralizing antibodies to the chemokine CXCL12 prevents recruitment of fibrocytes into the lung after bleomycin injury and reduces the amount of collagen accumulation by greater than 50% (134). Similarly, prevention of EMT has been reported to reduce bleomycin-induced pulmonary fibrosis (78).

Collagen Synthesis, Processing, and Structure

With the appropriate stimulation and environment, fibroblasts and myofibroblasts increase their production of collagen mRNA transcripts. Translation results in collagen propeptides that undergo modifications including hydroxylation of proline and lysine residues, glycosylation of lysine residues, and disulfide bond formation, ultimately leading to the formation of a triple helix consisting of three propeptides in the endoplasmic reticulum. Following packaging in the Golgi and secretion from the cell, peptidases further process collagen resulting in the mature monomeric collagen molecule. In type I collagen, five monomeric collagen molecules make up a microfibril that is staggered by a multiple of 67 nm, giving a characteristic banding pattern termed a D-period (71, 131, 183). Each microfibril interdigitates with neighboring microfibrils, creating a collagen fibril. There are both inter- and intramicrofibrillar cross-links mediated by aldehyde groups on lysine and hydroxylysine residues created by lysyl oxidase (125). A bundle of collagen fibrils then creates a collagen fiber (Fig. 2). The combination of extensive cross-linking and a right-handed

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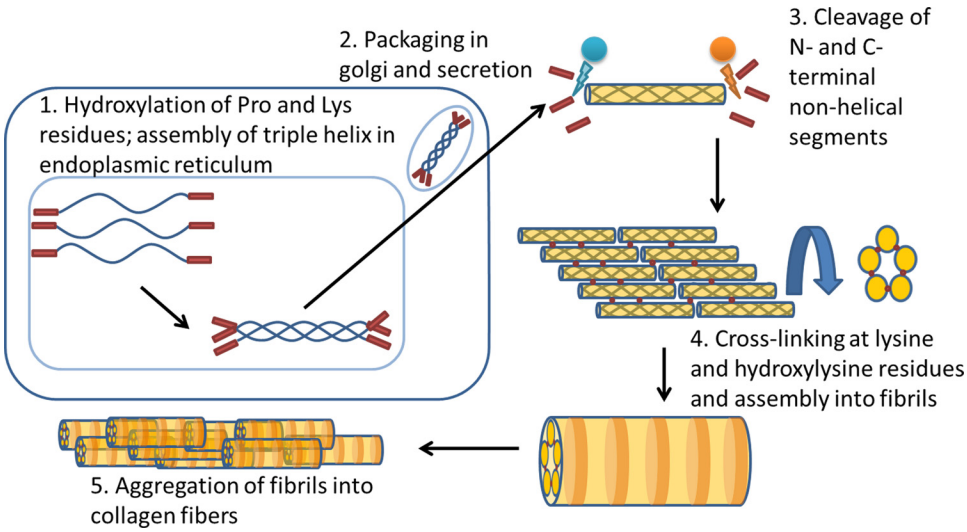
2. Packaging in golgi and secretion
3. Cleavage of N- and C-terminal non-helical segments
1. Hydroxylation of Pro and Lys residues; assembly of triple helix in endoplasmic reticulum
4. Cross-linking at lysine and hydroxylysine residues and assembly into fibrils
5. Aggregation of fibrils into collagen fibers

Fig. 2. Collagen synthesis begins with the translation of collagen pro-α chains in the rough endoplasmic reticulum. After these chains form a triple helix, the molecule is secreted from the cell, where it undergoes further processing and aggregation into fibrils and fibers.

supertwist structure of the microfibrils provides the tensile strength of fibrillar collagen (130). The intricacy of collagen synthesis is evident in scurvy, during which humans whose diets lack fruits and vegetables fail to develop scars or maintain connective tissue integrity. Proline hydroxylase requires ascorbic acid to properly hydroxylate proline into hydroxyproline. Hydroxyproline is required for proper stabilization of the triple helix structure of the collagen molecule. Thus a deficiency of ascorbic acid may promote the formation of partially hydroxyprolinated collagen that may be more prone to degradation (5, 72).

The structure and orientation of the collagen fibril is of particular importance when considering the interaction of collagen and its ligands in the ECM. Collagen binding sites have been identified for a number of molecules including integrins (76, 159), metalloproteinases (161), fibronectin (37), proteoglycans (146), and von Willebrand factor (144) among others. Many of these binding partners are critical for collagen fibril formation in vivo (74). Interestingly, some of these sites are buried within the predicted microfibrillar structure of collagen and are therefore not readily available for ligand interactions (131, 168). The fact that collagen recognition sites are not physically accessible to ligands suggests either that the structural predictions for collagen are not entirely correct or that collagen structure in vivo is fluid. The plasticity of collagen structure is supported by data suggesting that, at sites of collagenase cleavage, collagen exists in a partially unfolded conformation (124). Furthermore, at body temperature collagen may be thermodynamically unstable (94) and more readily available for proteolytic degradation. The accessibility of collagen degradation sites for proteolytic cleavage may be of particular importance in the resolution of fibrosis after injury. One possibility is that in areas of fibrosis collagen may primarily exist in a conformation that is less amenable to degradation.

Collagen Structure and Composition in Areas of Fibrosis

The composition of collagen is different in fibrotic tissue compared with normal tissue. The uninjured lung contains primarily type I and type III collagen with type I approximately twice as frequent as type III (62, 155). This relationship changes in lung fibrosis, favoring a predominance of type I collagen. A study examining the collagen composition of biopsy samples obtained from patients with a variety of fibrotic diseases found an increase in the proportion of type III collagen and an increase in total type I and type III collagen in early lung fibrosis. However, in more established areas of scar formation there was almost exclusively type I collagen (7). In studies conducted in the 1970s and 1980s of what was then called IPF (likely representing a mix of ILD pathologies with UIP predominating), the increase in collagen was found to be primarily an increase in type I collagen that represented ~80% of the total collagen (7, 101, 151, 155, 160). These studies indicate that in addition to an increase in total collagen in IPF (150, 151) there is also a shift toward the less elastic type I collagen, which contributes to pathophysiological abnormalities in fibrosis (51, 125, 145). A relative increase in type I collagen production (as well as an increase in total lung collagen content) has also been demonstrated in the lungs of patients with ARDS (87, 140) as well as in human fibrotic livers (85).

In addition to differences in the type of collagen that is expressed in scar tissue, there is an increase in production of other ECM molecules that intermingle with collagen (95). In liver fibrosis, for example, there is an increase in fibronectin, elastin, laminin, hyaluronan, chondroitin sulfate and heparin sulfate proteoglycans, and undulin in addition to increases in collagens (9, 60, 149). Similarly, in pulmonary fibrosis there is an increase in elastin, fibronectin, hyaluronan, and proteoglycans in addition to collagen (8, 39, 67, 163). This conglomeration of ECM molecules provides a unique ultrastructural challenge for matrix remodeling pathways and may prevent proteolytic enzymes from accessing their binding sites on collagen. Furthermore, some of these matrix molecules modulate the fibrotic process. Decorin, a small proteoglycan that has potentially opposing effects on fibrosis due to its dual roles in promoting fibril formation (184, 187) and suppressing the activity of the profibrotic cytokine TGF-β (80), is produced in excess by fibroblasts from patients with pulmonary fibrosis. And although TGF-β has been shown to reduce the production of decorin, long-standing connective tissue-associated fibrosis is associated with increased decorin production, which by promoting fibril formation may reduce collagen resorption.

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The importance of proteoglycans in modulating fibrosis is evident in data showing that intranasal installation of decorin in mice by viral delivery can prevent fibrosis induced by TGF-β (81) or bleomycin (80. Preferential production of fibrosis-promoting isoforms of ECM molecules is another mechanism by which altered ECM can exacerbate the fibrotic response. For example, the splice variant of fibronectin containing the extra type III domains (EDA) is induced by TGF-β (14) and promotes TGF-β-dependent induction of myofibroblasts and fibrosis (154). EDA is found interspersed between fibroblast and collagen in tissue sections from patients with IPF (83) and is produced in excess by fibroblasts from patients with IPF (121). EDA-null mice are protected from bleomycin-induced pulmonary fibrosis (121).

Collagen cross-linking by lysyl oxidase is another important structural modification that can prevent degradation in fibrosis. Lysyl oxidase expression and the proportion of hydroxylated lysine residues are increased in fibrotic states (104). An increase in cross-linking and altered cross-linking patterns have been implicated in dermal fibrosis (17), in ARDS in the lung (86), in myocardial fibrosis (98), and in pulmonary fibrosis (56, 142). A recent study demonstrated that inhibition of lysyl oxidase-like-2 prevented the development of bleomycin-induced lung fibrosis and reversed established fibrosis (6). Finally, collagen orientation in areas of fibrosis can also be altered. In skin scar tissue, collagen has a more parallel orientation relative to the epithelial surface (176, 177) compared with collagen in normal skin. Taken together, these data suggest that the type, hydroxylysine content, orientation, and cross-linking pattern of collagens as well as the molecules that interact with collagen differ in areas of fibrosis.

Collagen Turnover in the Lung

As outlined above, the appropriate stimulus leads to an increase in collagen production in the lung. However, not all collagen that is produced gets incorporated into fibrillar collagen. Newly synthesized collagen can be degraded prior to being secreted by the cell or prior to being incorporated into fibrils in the ECM (10, 12, 13). There is some evidence to suggest that with aging there is an increase in degradation of newly synthesized collagen prior to secretion from the cell and a decrease in degradation of extracellular collagen (108). Collagen that does become part of the ECM likely undergoes constant remodeling. Human lung explant studies demonstrated that adult lungs continuously produce collagen and the adult rat lung has been shown to undergo continuous collagen turnover (5, 15, 109, 126, 135), although other groups have used assays measuring labeled hydroxylysine cross-links to contend that there is very little or no degradation of collagen once it has been cross-linked and firmly incorporated into the ECM (88). More recent studies evaluating the kinetics of collagen metabolism have measured (by mass spectrometry) the incorporation of deuterium-labeled amino acids into collagen after replacing the drinking water of rodents with deuterium-labeled water (20, 54). This technique provides detailed data regarding the relative rates of collagen production and degradation in the resting state and after injury. Whereas the proportion of new collagen (collagen labeled with deuterium) after 14 days of deuterated water intake is roughly 10% in the normal adult lung, the proportion increases to ~40% after challenge with a dose of bleomycin that produces a 20% increase in total collagen (4). These studies delineate the dynamic turnover of collagen both in the normal lung and the lung after injury. What is unclear from this technique is whether all collagen in the lung is degraded at similar rates or whether there are pools of collagen that are more or less accessible to degradation (55, 58, 90). It has been suggested that the increased ratio of more stable dihydroxylysinonorleucine cross-links to less stable hydroxylysinonorleucine cross-links found in fibrotic lung tissue may protect "fibrotic collagen" from enzymatic degradation or mechanical damage (56). Further studies examining whether collagen that is incorporated into fibrillar scar is less prone to degradation than collagen that is part of normal tissue architecture are warranted.

Extracellular Pathway of Collagen Degradation

The molecular pathways responsible for collagen degradation have been incompletely described. Collagen metabolism is a two-step process that involves extracellular protease-mediated cleavage followed by cellular uptake and intracellular lysosomal degradation (43, 102, 161) (Fig. 3). Extracellular collagen degradation is mediated by recognition of specific cleavage sites that have characteristic imino acid content by matrix metalloproteinase enzymes (89, 166). Intact fibrillar collagen can only be cleaved by a subset of matrix metalloproteinases (MMPs) including MMP-1, MMP-8, MMP-13, MMP-14 (also known as MT1-MMP), MMP-16 (also known as MT3-MMP), and MMP-18 (161). Most MMPs consist of a catalytic domain and hemopexin domain. The hemopexin domain is thought to bind and unwind collagen, allowing access of the catalytic domain to the appropriate cleavage site. In fact, incubation of collagen with just the catalytic domain is inadequate for cleavage to take place (27, 28). Fragments of cleaved fibrillar collagen rapidly denature (i.e., lose their triple-helical structure) at 37°C and may then be further degraded by

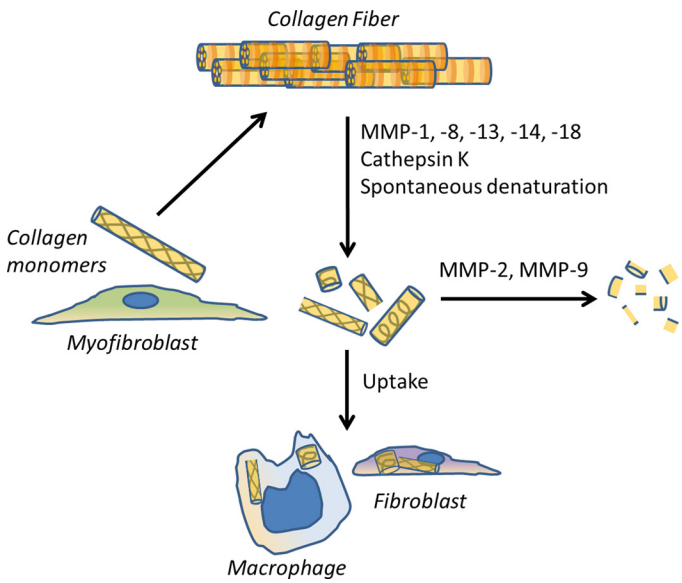

Fig. 3. Collagen monomers are incorporated into fibrils, which aggregate to form fibers. Degradation of collagen fibers involves cleavage of fibrils by collagenolytic enzymes and uptake of collagen fragments by macrophages and fibroblasts or further cleavage by gelatinases. MMP, matrix metalloproteinase.

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the gelatinases (MMP-2 and MMP-9). Collagen can also be cleaved by cathepsin K (18, 162), a lysosomal cysteine protease that is also active in the extracellular compartment. Cathepsin K is induced by inflammation (23) and cleaves type I and type II collagen both at the ends of the molecule and at multiple sites within the triple helix.

Several metalloproteinase enzymes, including MMP-1, 2, 3, 7, 9, and 14, are either highly expressed in the lungs of human patients with fibrosis (132) or have a defined role in experimental pulmonary fibrosis (132). The contribution of these enzymes to in vivo remodeling of fibrotic tissue, however, has been difficult to discern because of their multifunctional roles. For example, even though the expression of MMP-1, the prototypical metalloproteinase for degradation of fibrillar collagens, is increased in lungs of patients with IPF (50, 132), expression is restricted to the alveolar compartment rather than the interstitium. Increased expression of MMP-1 in fibrotic lungs may be related its role in alveolar reepithelialization rather than collagen degradation (42, 132). Furthermore, most experiments with MMP knockout mice show protection from bleomycin-induced fibrosis rather than increased collagen accumulation as might be expected from an in vivo defect in collagen degradation (Table 1). This is because, even though MMPs are proteolytic enzymes, their effects on targets other than collagens in vivo are greater determinants of their ultimate effects on the development of fibrosis. Although expression of both gelatinases (MMP-2 and MMP-9) is increased in pulmonary fibrosis, there is conflicting data regarding the consequences of gelatinase deficiency on the severity of experimentally induced fibrosis (11, 21, 129, 181) in the lung and other organs (64, 68, 107). A profibrotic role for these enzymes has been attributed to the fact that they mediate cleavage of basement membrane collagen during the acute phase of lung injury, allowing fibroblasts to migrate into the alveolar compartment and produce collagen leading to fibrosis (129, 167). MMP-3 expression is also increased in the lungs of patients with IPF, but it appears to promote fibrosis by promoting EMT (137, 185). MMP-3 overexpression in rats induces lung fibrosis and MMP-3 null mice are protected from bleomycin-induced pulmonary fibrosis. MMP-7 expression is also increased in IPF. As with MMP-3 and MMP-8 (53) knockout mice, MMP-7 knockout mice are protected from bleomycin-induced lung fibrosis (192).

Even when mice deficient in collagen-cleaving metalloproteinases accumulate interstitial collagens during development, they may be protected from fibrosis after injury. For example, MMP-13 knockout mice have defects in the development of bone and cartilage with accumulation of interstitial collagens (73). However, in a bile duct ligation model of liver fibrosis and in a lung model of radiation-induced fibrosis, MMP-13 knockouts are protected from developing fibrosis because of the effect of MMP-13 in regulating the initial inflammatory response (49, 173). In the carbon tetrachloride model of hepatic injury, MMP-13 knockouts developed the same initial degree of fibrosis but have a delay in resolution of fibrosis because of impaired collagen degradation (45). These conflicting results in different in vivo models of fibrosis emphasize the complex biological roles played by metalloproteinase enzymes.

The most convincing evidence for increased fibrosis resulting from a defect in extracellular collagen degradation comes from studies with MMP-14 and cathepsin K-deficient mice. MMP-14 knockout mice accumulate collagen extracellularly in the soft tissues (69, 92) and MMP-14 expression and activity is modulated by type I collagen (52, 191). Cathepsin K knockout mice develop more severe bleomycin-induced fibrosis and transgenic mice overexpressing cathepsin K have an abrogated response to bleomycin-induced fibrosis (18, 162). Additional support for an important role in ECM degradation comes from macrophage depletion studies. In carbon tetrachloride-induced liver fibrosis, the depletion of $CD11B^+$ cells after fibrosis is established prevents remodeling and resorption of fibrotic tissue (36) by decreasing local concentrations of MMP-13 secretion by scar-associated macrophages (45).

Table 1. *Experimental models of fibrosis in mice deficient in proteolytic enzymes*

Proteolytic Enzyme	Experimental Model	Results	Citation
MMP-2 KO	Cardiac pressure overload	Protection from fibrosis	107
MMP-2 KO	Myocardial infarction	Protection from fibrosis	64
MMP-2 KO	Diabetic nephropathy	Increased fibrosis	169
MMP-2 KO	CCl_4 liver fibrosis	Increased fibrosis	136
MMP-3 KO	Bleomycin lung fibrosis	Protection from fibrosis	185
MMP-3 overexpression	Transient adenoviral expression in rat lung	Induction of pulmonary fibrosis	185
MMP-7 KO	Bleomycin lung fibrosis	Protection from fibrosis	192
MMP-8 KO	Bleomycin lung fibrosis	Protection from fibrosis	53
MMP-9 KO	Renal obstruction	Protection from fibrosis	181
MMP-9 overexpression in macrophages	Bleomycin lung fibrosis	Protection from fibrosis	21
MMP-9 KO	OVA sensitization	Protection from fibrosis	96
MMP-9 KO	Cardiac pressure overload	Protection from fibrosis	68
MMP-13 KO	Normal development	Interstitial collagen accumulation	73
MMP-13 KO	Bile duct ligation	Protection from fibrosis	173
MMP-13 KO	CCl_4 liver fibrosis	Prolonged fibrosis	45
MMP-13 KO	Thoracic irradiation	Protection from fibrosis	49
MMP-13 KO	Lung hyperoxia	No change from WT	153
MMP-14 (MT1-MMP) KO	Normal development	Collagen accumulation in multiple tissues	69
Cathepsin K KO	Normal development	Increased fibrosis	186
Cathepsin K KO	Bleomycin lung fibrosis	Increased fibrosis	18
Cathepsin K overexpression	Bleomycin lung fibrosis	Protection from fibrosis	162
Neutrophil elastase KO	Bleomycin lung fibrosis	Protection from fibrosis	25

KO, knockout; WT, wild type; OVA, ovalbumin.

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The plasminogen activator system has recently been implicated in the resolution of fibrosis, and this effect may be mediated by modulation of extracellular collagen degradation pathways. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), which cleave plasminogen into active plasmin. PAI-1 is present in increased levels in human IPF samples (82) and is induced by TGF-β in an experimental mouse model of lung fibrosis (174). The PAI-1 knockout mouse is protected from lung (40) and kidney (127) fibrosis. One of the proposed mechanisms by which PAI-1 regulates fibrogenesis is by modulating activity of the proteinases necessary for ECM breakdown. Plasmin can directly or indirectly activate several matrix proteases, including pro-MMP-2, MMP-3, and MMP-9 (65, 117, 122, 141), as well as degrade ECM components present in fibrotic plaques including fibronectin and laminins (116). Furthermore, tPA has been shown to cause increased MMP-9 activity independent of plasmin, either by inducing release from neutrophils (32), by increasing MMP-9 transcription (189), or by liberating and activating ECM-bound HGF (63, 106), a potent antifibrotic growth factor that can cause upregulation of MMP-9 (57). Studies in lung (70) and liver (180) fibrosis models have shown that the profibrotic effect of PAI-1 depends on its inhibition of tPA or uPA and support the notion that impairment of normal extracellular collagen breakdown pathways can lead to pathologic fibrosis. However, another recent study on *PAI-1*$^{-/-}$ mice that had been transgenically engineered to selectively express PAI-1 protein constructs including or lacking PAI-1's antiprotease domain concluded that the major contribution of PAI-1 to fibrogenesis rests not on its antiproteolytic function but its ability to bind vitronectin (30). PAI-1 is clearly important in the development and resolution of fibrosis. However, like the metalloproteinases, its multiple functions make it difficult to precisely define its role in fibrosis.

Intracellular Pathway of Collagen Degradation

Compared with the pathways of extracellular collagen degradation, the pathways of intracellular collagen degradation, defined here as ingestion of collagen from the extracellular compartment, have been less extensively studied. Collagen phagocytosis by macrophages and fibroblasts was initially described by electron microscopy (35, 43, 99, 175). The identification of intracellular collagen fibrils in these studies argued against the possibility that intracellular staining represented newly synthesized collagen rather than collagen being internalized for degradation (41, 43). Studies of periodontal ligament fibroblasts indicated that internalized collagen is ultimately degraded in the lysosomal network (43) by cysteine proteinases (41, 42).

For collagen to be internalized it must first be recognized by specific cell surface receptors (Fig. 4). Collagen phagocytosis has been studied through the use of collagen-coated beads or native fibrillar collagen (156) and is dependent on the $\alpha_2\beta_1$ integrin (3, 24, 93). Fibroblasts using this integrin receptor bind and internalize collagen through a Gelsolin- and Rac-dependent pathway (2). A receptor-mediated endocytosis pathway of collagen uptake and intracellular degradation has been described for the transmembrane mannose receptors uPARAP/Endo180 (38) and mannose receptor/CD206 (105), both members of the mannose receptor family (103). The in vivo importance of uPARAP/Endo180-mediated collagen uptake is apparent in the increased collagen accumulation around areas of malignancy (33) as well as increased pulmonary fibrosis after treatment with bleomycin (111) in uPARAP/Endo180-knockout mice. The extracellular glycoprotein Mfge8 also mediates collagen uptake by binding collagen fragments and targeting them for uptake and digestion by macrophages. Mice deficient in Mfge8 have impaired collagen uptake and develop increased fibrosis after bleomycin injury because of an in vivo defect in collagen degradation (4). Additionally, a macropinocytic pathway of uptake has been reported for dendritic cell and macrophage-mediated uptake of collagen in studies using pharmacological inhibitors (179).

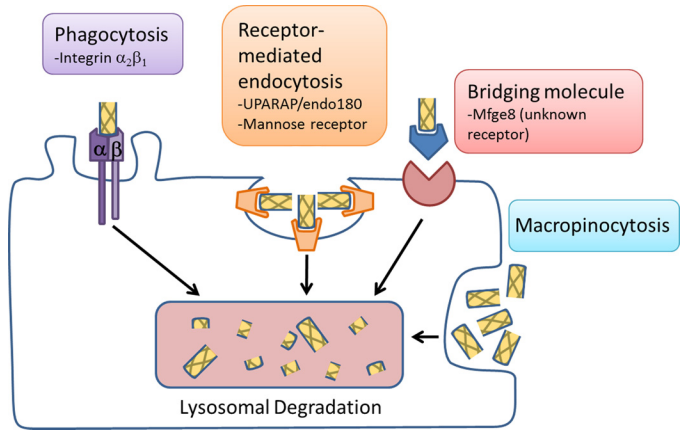

Fig. 4. Collagen internalization is mediated by macropinocytic, phagocytic, and endocytic pathways. Multiple cell-surface receptors have been shown to bind collagen, but the majority of those act to tether the cell to the ECM or relay signals from the ECM to the intracellular compartment. The receptors shown above have been shown to specifically mediate internalization of bound collagen fragments.

Given the importance of inflammation in injury and repair, it is not surprising that, at least in vitro, the intracellular pathways of collagen degradation are modulated by cytokines. In in vitro assays, the role of the critical cytokines TNF-α, TGF-β, and IL-1α on collagen uptake have been examined. TGF-β, generally considered an anti-inflammatory cytokine, augments collagen phagocytosis by periosteal fibroblasts and inhibits collagenase release (175). TNF-α and IL-1α, both early proinflammatory cytokines, inhibit collagen uptake and induce MMP release/activity (24, 44, 158, 175). Since uncleared collagen fragments are thought to be proinflammatory (143, 182), it is possible that one mechanism by which these cytokines regulate inflammation is through removal of collagen fragments from the extracellular compartment. The effect of inflammation on the intracellular collagen degradation pathway in vivo has not been evaluated experimentally.

Consequences of Impaired Extra- or Intracellular Degradation Pathways on Fibrosis in Humans

Though increased collagenase expression has been reported in both human and experimental murine models of pulmonary fibrosis (66, 75, 192), there is some evidence for impaired extracellular collagen degrading capacity in fibrotic tissue. In human patients with IPF there is an increase in the ratio of tissue inhibitors of metalloproteinases (TIMP) to collagenases

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(118, 152) evaluated by immunohistochemistry of tissue sections and decreased collagenolytic activity in lung homogenates (151). Similarly, in a rat model of experimental lung silicosis, there is a relative increase in TIMP-1 and TIMP-2 relative to MMP expression by immunohistochemistry and in situ hybridization in late silicotic granulomas (133). A decrease in collagenolytic activity has also been reported in cirrhotic livers (119, 128) and in the skin of patients with scleroderma (16). Patients with acute lung injury are at risk of developing lung fibrosis have and been reported to have reduced lung collagenolytic activity. One study of patients with ARDS and acute lung injury measured the ratio of the COOH-terminal propeptide of type I collagen (a marker of collagen synthesis) to the carboxy terminal sequence of the 3/4 degradation fragment of type I collagen (a marker of collagen degradation). The authors found that patients with more severe lung injury had less total and relative quantities of degraded collagen fragments and higher levels of collagen propeptide, indicating an in vivo defect in collagen degradation (1). Taken together, these data suggest that in areas of fibrosis there is inhibition of the pathways that mediate extracellular collagen degradation. It is unclear how this occurs. One possibility is that even though there are a large number of fibroblasts and macrophages in fibrotic tissue, these cells have altered profiles of collagenase and TIMP expression due to cues provided by the fibrotic microenvironment surrounding them.

The first evidence that a defect in the intracellular degradation pathway can lead to an increase in extracellular collagen accumulation comes from studies of patients who develop gingival hyperplasia as an adverse reaction to drug therapy with medications such as cyclosporine, dilantin, and nifedipine (22, 110, 112). Cyclosporine induces accumulation of subepithelial collagen in part by inhibiting integrin-mediated release of intracellular calcium stores that are required for collagen binding prior to phagocytosis (22, 34). Human gingival fibroblasts isolated from fibrotic lesions have impaired collagen bead phagocytosis that can be replicated by incubation of normal fibroblasts in vitro with dilantin or nifedipine (111). A similar defect in fibroblast-mediated collagen phagocytosis is thought to occur in the chronic mucosal condition oral submucous fibrosis (OSF). OSF lesions are characterized by an increase in collagen deposition in the subepithelial layer of the oral mucosa. In vitro evidence suggests that fibroblasts cultured from patients with OSF have increased production and impaired uptake of collagen (170). Incubation of normal fibroblasts with areca nut alkaloids, the putative toxin that causes OSF, inhibits collagen phagocytosis, and this effect is reversed by treatment with corticosteroids (157, 172) (Table 2).

Relationship Between Extracellular and Intracellular Degradation Pathways

One interesting area of active investigation is focused on understanding the relationship between the extracellular and intracellular degradation pathways in vivo. The logical assumption has been that these pathways function in a serial fashion with extracellular degradation of fibrillar collagen preceding cellular uptake and intracellular degradation. Since the collagen fibril is much larger than a single cell it would seem absolutely necessary for the fibril to be partially degraded prior to uptake. Although some studies do not support this model (24, 42, 44), there is compelling evidence that, at least in vitro, uptake of collagen proceeds much more efficiently after metalloproteinase-mediated cleavage (92, 102) and denaturation of the collagen fragments. The most satisfying model is one where cells that take up collagen for intracellular degradation express metalloproteinases in a geographically restricted manner at areas of contact with collagen (43, 84, 92). This allows for local proteolytic cleavage of fibrillar collagen while avoiding unrestricted exposure of normal tissue to increased collagenase levels. Cells can then take up collagen for further intracellular degradation by either phagocytosis, receptor-mediated endocytosis, or macropinocytosis. One might wonder why uptake of cleaved collagen is necessary since gelatinase enzymes can further degrade fragments in the extracellular space. However, the orderly removal of collagen fragments is important for preventing both acute and chronic inflammation. Cleaved collagen fragments can induce acute inflammation through neutrophil recruitment (143, 182) and can serve an antigens inducing autoimmunity (148, 171).

Assuming that this model is correct, a provocative question is why a defect in intracellular degradation would lead to increased extracellular accumulation of collagen. One attractive hypothesis is that the binding of collagen by cell surface receptors or subsequent internalization can regulate the production and secretion of extracellular proteolytic enzymes. Therefore, an increase in intracellular collagen or cell surface binding of collagen would signal to the cell to increase its production of collagenases and a subsequent decrease in intracellular collagen or cell surface binding of collagen would decrease collagenase production. In this model, the uptake of collagen for intracellular degradation could regulate extracel-

Table 2. *Evidence for impaired collagen degradation leading to fibrosis in vivo*

Experimental Population	Mechanism of Impaired Degradation	Citation
Patients with IPF/UIP	Increase ratio of TIMPs to collagenases	118, 152
	Decreased collagenolytic activity	151
Patients with scleroderma	Decreased collagenolytic activity	16
Patients with drug-induced gingival hyperplasia	Subepithelial collagen accumulation with associated impaired collagen binding/phagocytosis by fibroblasts	22, 113, 34, 111
Patients with oral submucous fibrosis	Subepithelial collagen accumulation with reduced collagen phagocytosis by fibroblasts	157, 172
Patients with ARDS/ALI	Decreased degradation of type I collagen in vivo with more severe disease	1
Exaggerated bleomycin-induced lung fibrosis in mice	Collagen accumulation due to impaired collagen binding/phagocytosis	4, 19

IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonitis; ARDS, acute respiratory distress syndrome; ALI, acute lung injury; TIMPs, tissue inhibitors of metalloproteinases.

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lular cleavage by regulating proteolytic enzyme production. Although there are no data supporting this hypothesis, there is some work to suggest the opposite. uPARAP/Endo180-mediated collagen uptake inhibits the cell surface activity level of MMP-14 uptake, therefore decreasing the collagenase activity and preventing MMP-14 induced cleavage and activation of the gelatinase MMP-2 (115). As discussed above, TNF-α, TGF-β, and IL-1α have reciprocal effects on collagen phagocytosis and collagenase activity (24).

To further complicate matters, some evidence suggests that a defect in extracellular cleavage of collagen can lead to an increase in the activity of collagen uptake and intracellular degradation. Deficiency of MMP-14 leads to an increase in extracellular collagen accumulation (147) but is also associated with a large increase in intracellular collagen accumulation (69), suggesting that a compensatory increase in intracellular degradation may occur with defective extracellular degradation. There is also some evidence that the extracellular or intracellular pathway may be more active under different experimental conditions. For example, in animal models of emphysema, the extracellular pathway of collagen degradation predominates when levels of collagenolytic elastases (26) are high, whereas the intracellular pathway predominates when the development of emphysema is not associated with a high elastase burden (99). It is unclear whether these differences are due to collagen cleavage by elastase or mediated by its interactions with MMPs and/or TIMPs.

Summary and Conclusions

Collagen is the most abundant protein in our bodies and its production and tissue deposition are exquisitely regulated to maintain tissue homeostasis. Collagen is continuously produced and degraded, and under homeostatic conditions the rate of production and degradation are perfectly matched to prevent the development of fibrosis with subsequent organ dysfunction. With tissue injury there is an appropriate increase in collagen production to form a provisional matrix and allow for tissue repair. Injury also induces an increase in collagen degradation pathways that remodel areas of fibrosis. These pathways in the lung can lead to the restoration of normal or near-normal tissue architecture with the ability to engage in efficient gas exchange. Despite the presence of these degradation pathways, tissue injury can lead to unresolved and sometimes progressive fibrosis. Whether the development of permanent fibrosis requires a failure of collagen degradation is unknown although there is some evidence of inhibition of degradation pathways in pulmonary fibrosis.

Although no therapies currently exist that effectively treat pulmonary fibrosis, the majority of scientific effort has been and continues to be directed toward understanding the pathways that lead to excess collagen production with the goal of inhibiting these pathways to prevent the development of fibrosis. In UIP, the most vexing disease from a treatment and prognosis perspective, most patients have a significant fibrotic burden at the time of initial diagnosis and would benefit greatly from therapies that increase collagen degradation. If we accept that there is a need for therapies that promote resolution of established fibrosis in addition to those that prevent collagen production, we must consider what the most promising avenues are for future investigation. The use of proteolytic enzymes to augment ECM degradation is fraught with potentially disastrous adverse effects due to uncontrolled parenchymal destruction of normal and abnormal tissue and is therefore not a realistic option. The use of inhibitors of collagen cross-linking is a much more promising approach in that it may allow endogenous proteolytic enzymes to access previously hidden degradation sites on collagen molecules. Additionally, these inhibitors may decrease the synthesis of highly cross-linked and stable collagen and thus allow more rapid cleavage of less cross-linked and less stable collagen. Ideally, this will lead to gradual and persistent degradation of fibrillar scar without having a significant impact on collagens that are part of the normal tissue architecture. The ability of an inhibitor of lysyl oxidase-like-2 to reverse established bleomycin-induced lung fibrosis in mice (6) highlights the importance of further treatments aimed at preventing the structural changes that make collagen resistant to degradation in fibrotic tissue. Another area that warrants further evaluation is examining whether therapies that augment intracellular pathways of collagen degradation will lead to enhanced extracellular remodeling of fibrotic tissue. There is now convincing evidence that defective intracellular collagen degradation leads to excess fibrosis in experimental mouse models (4, 19). These data show that inhibition of key pathways mediating collagen uptake by cells promote extracellular accumulation of fibrillar collagen. What remains to be established is whether there are cellular pathways that inhibit uptake of collagen for intracellular degradation. If so, antagonizing these inhibitory pathways may increase collagen uptake and degradation and optimally lead to enhanced resorption of extracellular fibrillar collagens in areas of fibrosis. Even if these therapies do not restore normal gas exchange, the reduction in scar tissue may improve lung mechanics and oppose the increase in parenchymal stiffness that can further activate collagen producing pathways (97, 190). Despite extensive research, there is still much to be learned about the structural modifications that prevent breakdown of scar-associated collagen and the interplay between the intracellular and extracellular pathways of collagen degradation. A better understanding of these processes may lead to novel therapies for the treatment of pulmonary fibrosis.

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AUTHOR CONTRIBUTIONS

W.M. and K.A. prepared figures; W.M., T.-H.L., and K.A. drafted manuscript; W.M. and K.A. edited and revised manuscript; K.A. conception and design of research; K.A. approved final version of manuscript.

REFERENCES

1. **Armstrong L, Thickett DR, Mansell JP, Ionescu M, Hoyle E, Billinghurst RC, Poole AR, Millar AB.** Changes in collagen turnover in early acute respiratory distress syndrome. *Am J Respir Crit Care Med* 160: 1910–1915, 1999.
2. **Arora PD, Glogauer M, Kapus A, Kwiatkowski DJ, McCulloch CA.** Gelsolin mediates collagen phagocytosis through a rac-dependent step. *Mol Biol Cell* 15: 588–599, 2004.
3. **Arora PD, Manolson MF, Downey GP, Sodek J, McCulloch CA.** A novel model system for characterization of phagosomal maturation,

Downloaded from journals.physiology.org/journal/ajplung (038.207.136.179) on October 24, 2025.

acidification, and intracellular collagen degradation in fibroblasts. *J Biol Chem* 275: 35432–35441, 2000.

4. **Atabai K, Jame S, Azhar N, Kuo A, Lam M, McKleroy W, Dehart G, Rahman S, Xia DD, Melton AC, Wolters P, Emson CL, Turner SM, Werb Z, Sheppard D.** Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. *J Clin Invest* 119: 3713–3722, 2009.
5. **Barnes MJ, Constable BJ, Morton LF, Kodicek E.** Studies in vivo on the biosynthesis of collagen and elastin in ascorbic acid-deficient guinea pigs. Evidence for the formation and degradation of a partially hydroxylated collagen. *Biochem J* 119: 575–585, 1970.
6. **Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, Mikels A, Vaysberg M, Ghermazien H, Wai C, Garcia CA, Velayo AC, Jorgensen B, Biermann D, Tsai D, Green J, Zaffryar-Eilot S, Holzer A, Ogg S, Thai D, Neufeld G, Van Vlasselaer P, Smith V.** Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat Med* 16: 1009–1017, 2010.
7. **Bateman ED, Turner-Warwick M, Adelmann-Grill BC.** Immunohistochemical study of collagen types in human foetal lung and fibrotic lung disease. *Thorax* 36: 645–653, 1981.
8. **Bensadoun ES, Burke AK, Hogg JC, Roberts CR.** Proteoglycan deposition in pulmonary fibrosis. *Am J Respir Crit Care Med* 154: 1819–1828, 1996.
9. **Benyon RC, Iredale JP.** Is liver fibrosis reversible? *Gut* 46: 443–446, 2000.
10. **Berg RA, Schwartz ML, Crystal RG.** Regulation of the production of secretory proteins: intracellular degradation of newly synthesized "defective" collagen. *Proc Natl Acad Sci USA* 77: 4746–4750, 1980.
11. **Betsuyaku T, Fukuda Y, Parks WC, Shipley JM, Senior RM.** Gelatinase B is required for alveolar bronchiolization after intratracheal bleomycin. *Am J Pathol* 157: 525–535, 2000.
12. **Bienkowski RS.** Intracellular degradation of newly synthesized collagen. *Revis Biol Celular* 21: 423–443, 1989.
13. **Bienkowski RS, Baum BJ, Crystal RG.** Fibroblasts degrade newly synthesised collagen within the cell before secretion. *Nature* 276: 413–416, 1978.
14. **Borsi L, Castellani P, Risso AM, Leprini A, Zardi L.** Transforming growth factor-beta regulates the splicing pattern of fibronectin messenger RNA precursor. *FEBS Lett* 261: 175–178, 1990.
15. **Bradley K, McConnell-Breul S, Crystal RG.** Collagen in the human lung. Quantitation of rates of synthesis and partial characterization of composition. *J Clin Invest* 55: 543–550, 1975.
16. **Brady AH.** Collagenase in scleroderma. *J Clin Invest* 56: 1175–1180, 1975.
17. **Brinckmann J, Neess CM, Gaber Y, Sobhi H, Notbohm H, Hunzelmann N, Fietzek PP, Muller PK, Risteli J, Gebker R, Scharffetter-Kochanek K.** Different pattern of collagen cross-links in two sclerotic skin diseases: lipodermatosclerosis and circumscribed scleroderma. *J Invest Dermatol* 117: 269–273, 2001.
18. **Buhling F, Rocken C, Brasch F, Hartig R, Yasuda Y, Saftig P, Bromme D, Welte T.** Pivotal role of cathepsin K in lung fibrosis. *Am J Pathol* 164: 2203–2216, 2004.
19. **Bundesmann MM, Wagner TE, Chow YH, Altemeier WA, Steinbach T, Schnapp LM.** Role of urokinase plasminogen activator receptor associated protein (uPARAP) in mouse lung. *Am J Respir Cell Mol Biol* 46: 233–239, 2012.
20. **Busch R, Kim YK, Neese RA, Schade-Serin V, Collins M, Awada M, Gardner JL, Beysen C, Marino ME, Misell LM, Hellerstein MK.** Measurement of protein turnover rates by heavy water labeling of nonessential amino acids. *Biochim Biophys Acta* 1760: 730–744, 2006.
21. **Cabrera S, Gaxiola M, Arreola JL, Ramirez R, Jara P, D'Armiento J, Richards T, Selman M, Pardo A.** Overexpression of MMP9 in macrophages attenuates pulmonary fibrosis induced by bleomycin. *Int J Biochem Cell Biol* 39: 2324–2338, 2007.
22. **Chan MW, Arora PD, McCulloch CA.** Cyclosporin inhibition of collagen remodeling is mediated by gelsolin. *Am J Physiol Cell Physiol* 293: C1049–C1058, 2007.
23. **Chapman HA, Riese RJ, Shi GP.** Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol* 59: 63–88, 1997.
24. **Chou DH, Lee W, McCulloch CA.** TNF-alpha inactivation of collagen receptors: implications for fibroblast function and fibrosis. *J Immunol* 156: 4354–4362, 1996.
25. **Chua F, Dunsmore SE, Clingen PH, Mutsaers SE, Shapiro SD, Segal AW, Roes J, Laurent GJ.** Mice lacking neutrophil elastase are resistant to bleomycin-induced pulmonary fibrosis. *Am J Pathol* 170: 65–74, 2007.
26. **Chua F, Laurent GJ.** Neutrophil elastase: mediator of extracellular matrix destruction and accumulation. *Proc Am Thorac Soc* 3: 424–427, 2006.
27. **Chung L, Dinakarpandian D, Yoshida N, Lauer-Fields JL, Fields GB, Visse R, Nagase H.** Collagenase unwinds triple-helical collagen prior to peptide bond hydrolysis. *EMBO J* 23: 3020–3030, 2004.
28. **Clark IM, Cawston TE.** Fragments of human fibroblast collagenase. Purification and characterization. *Biochem J* 263: 201–206, 1989.
29. **Coalson JJ.** The ultrastructure of human fibrosing alveolitis. *Virchows Arch A Pathol Anat Histol* 395: 181–199, 1982.
30. **Courey AJ, Horowitz JC, Kim KK, Koh TJ, Novak ML, Subbotina N, Warnock M, Xue B, Cunningham AK, Lin Y, Goldklang MP, Simon RH, Lawrence DA, Sisson TH.** The vitronectin-binding function of PAI-1 exacerbates lung fibrosis in mice. *Blood* 118: 2313–2321, 2011.
31. **Coward WR, Saini G, Jenkins G.** The pathogenesis of idiopathic pulmonary fibrosis. *Ther Adv Respir Dis* 4: 367–388, 2010.
32. **Cuadrado E, Ortega L, Hernandez-Guillamon M, Penalba A, Fernandez-Cadenas I, Rosell A, Montaner J.** Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. *J Leukoc Biol* 84: 207–214, 2008.
33. **Curino AC, Engelholm LH, Yamada SS, Holmbeck K, Lund LR, Molinolo AA, Behrendt N, Nielsen BS, Bugge TH.** Intracellular collagen degradation mediated by uPARAP/Endo180 is a major pathway of extracellular matrix turnover during malignancy. *J Cell Biol* 169: 977–985, 2005.
34. **Deliliers GL, Santoro F, Polli N, Bruno E, Fumagalli L, Risciotti E.** Light and electron microscopic study of cyclosporin A-induced gingival hyperplasia. *J Periodontol* 57: 771–775, 1986.
35. **Deporter DA.** Collagen phagocytosis by stimulated mouse peritoneal macrophages in vitro. *J Periodontal Res* 14: 323–331, 1979.
36. **Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP.** Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* 115: 56–65, 2005.
37. **Dzamba BJ, Wu H, Jaenisch R, Peters DM.** Fibronectin binding site in type I collagen regulates fibronectin fibril formation. *J Cell Biol* 121: 1165–1172, 1993.
38. **East L, McCarthy A, Wienke D, Sturge J, Ashworth A, Isacke CM.** A targeted deletion in the endocytic receptor gene Endo180 results in a defect in collagen uptake. *EMBO Rep* 4: 710–716, 2003.
39. **Ebihara T, Venkatesan N, Tanaka R, Ludwig MS.** Changes in extracellular matrix and tissue viscoelasticity in bleomycin-induced lung fibrosis. Temporal aspects. *Am J Respir Crit Care Med* 162: 1569–1576, 2000.
40. **Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D, Simon RH.** Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 97: 232–237, 1996.
41. **Everts V, Beertsen W, Tigchelaar-Gutter W.** The digestion of phagocytosed collagen is inhibited by the proteinase inhibitors leupeptin and E-64. *Coll Relat Res* 5: 315–336, 1985.
42. **Everts V, Hembry RM, Reynolds JJ, Beertsen W.** Metalloproteinases are not involved in the phagocytosis of collagen fibrils by fibroblasts. *Matrix* 9: 266–276, 1989.
43. **Everts V, van der Zee E, Creemers L, Beertsen W.** Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. *Histochem J* 28: 229–245, 1996.
44. **Everts V, Wolvius E, Saklatvala J, Beertsen W.** Interleukin 1 increases the production of collagenase but does not influence the phagocytosis of collagen fibrils. *Matrix* 10: 388–393, 1990.
45. **Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, Iredale JP.** Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol* 178: 5288–5295, 2007.
46. **Fernandez IE, Eickelberg O.** New cellular and molecular mechanisms of lung injury and fibrosis in idiopathic pulmonary fibrosis. *Lancet* 380: 680–688, 2012.
47. **Fine A, Goldstein RH.** The effect of transforming growth factor-beta on cell proliferation and collagen formation by lung fibroblasts. *J Biol Chem* 262: 3897–3902, 1987.

Downloaded from journals.physiology.org/journal/ajplung (038.207.136.179) on October 24, 2025.

48. **Fischer A, du Bois R.** Interstitial lung disease in connective tissue disorders. *Lancet* 380: 689–698, 2012.
49. **Flechsig P, Hartenstein B, Teurich S, Dadrich M, Hauser K, Abdollahi A, Grone HJ, Angel P, Huber PE.** Loss of matrix metalloproteinase-13 attenuates murine radiation-induced pulmonary fibrosis. *Int J Radiat Oncol Biol Phys* 77: 582–590, 2010.
50. **Fukuda Y, Ishizaki M, Kudoh S, Kitaichi M, Yamanaka N.** Localization of matrix metalloproteinases-1, -2, and -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. *Lab Invest* 78: 687–698, 1998.
51. **Fulmer JD, Bienkowski RS, Cowan MJ, Breul SD, Bradley KM, Ferrans VJ, Roberts WC, Crystal RG.** Collagen concentration and rates of synthesis in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 122: 289–301, 1980.
52. **Galvez BG, Matias-Roman S, Yanez-Mo M, Sanchez-Madrid F, Arroyo AG.** ECM regulates MT1-MMP localization with beta1 or alphavbeta3 integrins at distinct cell compartments modulating its internalization and activity on human endothelial cells. *J Cell Biol* 159: 509–521, 2002.
53. **Garcia-Prieto E, Gonzalez-Lopez A, Cabrera S, Astudillo A, Gutierrez-Fernandez A, Fanjul-Fernandez M, Batalla-Solis E, Puente XS, Fueyo A, Lopez-Otin C, Albaiceta GM.** Resistance to bleomycin-induced lung fibrosis in MMP-8 deficient mice is mediated by interleukin-10. *PloS One* 5: e13242, 2010.
54. **Gardner JL, Turner SM, Bautista A, Lindwall G, Awada M, Hellerstein MK.** Measurement of liver collagen synthesis by heavy water labeling: effects of profibrotic toxicants and antifibrotic interventions. *Am J Physiol Gastrointest Liver Physiol* 292: G1695–G1705, 2007.
55. **Gerber G, Gerber G, Altman KI.** Studies on the metabolism of tissue proteins. I. Turnover of collagen labeled with proline-U-C14 in young rats. *J Biol Chem* 235: 2653–2656, 1960.
56. **Gerriets JE, Reiser KM, Last JA.** Lung collagen cross-links in rats with experimentally induced pulmonary fibrosis. *Biochim Biophys Acta* 1316: 121–131, 1996.
57. **Gong R, Rifai A, Tolbert EM, Centracchio JN, Dworkin LD.** Hepatocyte growth factor modulates matrix metalloproteinases and plasminogen activator/plasmin proteolytic pathways in progressive renal interstitial fibrosis. *J Am Soc Nephrol* 14: 3047–3060, 2003.
58. **Green NM, Lowther DA.** Formation of collagen hydroxyproline in vitro. *Biochem J* 71: 55–66, 1959.
59. **Gross TJ, Hunninghake GW.** Idiopathic pulmonary fibrosis. *N Engl J Med* 345: 517–525, 2001.
60. **Hahn E, Wick G, Pencev D, Timpl R.** Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin, and fibronectin. *Gut* 21: 63–71, 1980.
61. **Hamutcu R, Rowland JM, Horn MV, Kaminsky C, MacLaughlin EF, Starnes VA, Woo MS.** Clinical findings and lung pathology in children with cystic fibrosis. *Am J Respir Crit Care Med* 165: 1172–1175, 2002.
62. **Hance AJ, Crystal RG.** The connective tissue of lung. *Am Rev Respir Dis* 112: 657–711, 1975.
63. **Hattori N, Mizuno S, Yoshida Y, Chin K, Mishima M, Sisson TH, Simon RH, Nakamura T, Miyake M.** The plasminogen activation system reduces fibrosis in the lung by a hepatocyte growth factor-dependent mechanism. *Am J Pathol* 164: 1091–1098, 2004.
64. **Hayashidani S, Tsutsui H, Ikeuchi M, Shiomi T, Matsusaka H, Kubota T, Imanaka-Yoshida K, Itoh T, Takeshita A.** Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am J Physiol Heart Circ Physiol* 285: H1229–H1235, 2003.
65. **He CS, Wilhelm SM, Pentland AP, Marmer BL, Grant GA, Eisen AZ, Goldberg GI.** Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc Natl Acad Sci USA* 86: 2632–2636, 1989.
66. **Henry MT, McMahon K, Mackarel AJ, Prikk K, Sorsa T, Maisi P, Sepper R, Fitzgerald MX, O'Connor CM.** Matrix metalloproteinases and tissue inhibitor of metalloproteinase-1 in sarcoidosis and IPF. *Eur Respir J* 20: 1220–1227, 2002.
67. **Hernnas J, Nettelbladt O, Bjermer L, Sarnstrand B, Malmstrom A, Hallgren R.** Alveolar accumulation of fibronectin and hyaluronan precedes bleomycin-induced pulmonary fibrosis in the rat. *Eur Respir J* 5: 404–410, 1992.
68. **Heymans S, Lupu F, Terclavers S, Vanwetswinkel B, Herbert JM, Baker A, Collen D, Carmeliet P, Moons L.** Loss or inhibition of uPA or MMP-9 attenuates LV remodeling and dysfunction after acute pressure overload in mice. *Am J Pathol* 166: 15–25, 2005.
69. **Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H.** MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* 99: 81–92, 1999.
70. **Huang WT, Vayalil PK, Miyata T, Hagood J, Liu RM.** Therapeutic value of small molecule inhibitor to plasminogen activator inhibitor-1 for lung fibrosis. *Am J Respir Cell Mol Biol* 46: 87–95, 2012.
71. **Hulmes DJ, Jesior JC, Miller A, Berthet-Colominas C, Wolff C.** Electron microscopy shows periodic structure in collagen fibril cross sections. *Proc Natl Acad Sci USA* 78: 3567–3571, 1981.
72. **Hurych J, Chvapil M, Tichy M, Beniac F.** Evidence for a fastr degradation of an atypical hydroxyproline and hydroxylysine deficient collagen formed under the effect of 2,2′-dipyridyl. *Eur J Biochem* 3: 242–247, 1967.
73. **Inada M, Wang Y, Byrne MH, Rahman MU, Miyaura C, Lopez-Otin C, Krane SM.** Critical roles for collagenase-3 (Mmp13) in development of growth plate cartilage and in endochondral ossification. *Proc Natl Acad Sci USA* 101: 17192–17197, 2004.
74. **Kadler KE, Hill A, Canty-Laird EG.** Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr Opin Cell Biol* 20: 495–501, 2008.
75. **Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA.** Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci USA* 97: 1778–1783, 2000.
76. **Kapyla J, Ivaska J, Riikonen R, Nykvist P, Pentikainen O, Johnson M, Heino J.** Integrin alpha(2)I domain recognizes type I and type IV collagens by different mechanisms. *J Biol Chem* 275: 3348–3354, 2000.
77. **Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA.** Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* 103: 13180–13185, 2006.
78. **Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, Frank JA, Brumwell AN, Wheeler SE, Kreidberg JA, Chapman HA.** Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J Clin Invest* 119: 213–224, 2009.
79. **King TE Jr, Pardo A, Selman M.** Idiopathic pulmonary fibrosis. *Lancet* 378: 1949–1961, 2011.
80. **Kolb M, Margetts PJ, Galt T, Sime PJ, Xing Z, Schmidt M, Gauldie J.** Transient transgene expression of decorin in the lung reduces the fibrotic response to bleomycin. *Am J Respir Crit Care Med* 163: 770–777, 2001.
81. **Kolb M, Margetts PJ, Sime PJ, Gauldie J.** Proteoglycans decorin and biglycan differentially modulate TGF-beta-mediated fibrotic responses in the lung. *Am J Physiol Lung Cell Mol Physiol* 280: L1327–L1334, 2001.
82. **Kotani I, Sato A, Hayakawa H, Urano T, Takada Y, Takada A.** Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary fibrosis. *Thromb Res* 77: 493–504, 1995.
83. **Kuhn C, McDonald JA.** The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am J Pathol* 138: 1257–1265, 1991.
84. **Lafleur MA, Mercuri FA, Ruangpanit N, Seiki M, Sato H, Thompson EW.** Type I collagen abrogates the clathrin-mediated internalization of membrane type 1 matrix metalloproteinase (MT1-MMP) via the MT1-MMP hemopexin domain. *J Biol Chem* 281: 6826–6840, 2006.
85. **Lai KK, Shang S, Lohia N, Booth GC, Masse DJ, Fausto N, Campbell JS, Beretta L.** Extracellular matrix dynamics in hepatocarcinogenesis: a comparative proteomics study of PDGFC transgenic and Pten null mouse models. *PLoS Genet* 7: e1002147.
86. **Last JA, King TE Jr, Nerlich AG, Reiser KM.** Collagen cross-linking in adult patients with acute and chronic fibrotic lung disease. Molecular markers for fibrotic collagen. *Am Rev Respir Dis* 141: 307–313, 1990.
87. **Last JA, Siefkin AD, Reiser KM.** Type I collagen content is increased in lungs of patients with adult respiratory distress syndrome. *Thorax* 38: 364–368, 1983.
88. **Last JA, Summers P, Reiser KM.** Biosynthesis of collagen crosslinks. II. In vivo labelling and stability of lung collagen in rats. *Biochim Biophys Acta* 990: 182–189, 1989.

Downloaded from journals.physiology.org/journal/ajplung (038.207.136.179) on October 24, 2025.

89. **Lauer-Fields JL, Juska D, Fields GB.** Matrix metalloproteinases and collagen catabolism. *Biopolymers* 66: 19–32, 2002.
90. **Laurent GJ.** Dynamic state of collagen: pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. *Am J Physiol Cell Physiol* 252: C1–C9, 1987.
91. **Laurent GJ.** Lung collagen: more than scaffolding. *Thorax* 41: 418–428, 1986.
92. **Lee H, Overall CM, McCulloch CA, Sodek J.** A critical role for the membrane-type 1 matrix metalloproteinase in collagen phagocytosis. *Mol Biol Cell* 17: 4812–4826, 2006.
93. **Lee W, Sodek J, McCulloch CA.** Role of integrins in regulation of collagen phagocytosis by human fibroblasts. *J Cell Physiol* 168: 695–704, 1996.
94. **Leikina E, Mertts MV, Kuznetsova N, Leikin S.** Type I collagen is thermally unstable at body temperature. *Proc Natl Acad Sci USA* 99: 1314–1318, 2002.
95. **Li Y, Jiang D, Liang J, Meltzer EB, Gray A, Miura R, Wogensen L, Yamaguchi Y, Noble PW.** Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med* 208: 1459–1471, 2011.
96. **Lim DH, Cho JY, Miller M, McElwain K, McElwain S, Broide DH.** Reduced peribronchial fibrosis in allergen-challenged MMP-9-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 291: L265–L271, 2006.
97. **Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ.** Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* 190: 693–706, 2010.
98. **Lopez B, Gonzalez A, Hermida N, Valencia F, de Teresa E, Diez J.** Role of lysyl oxidase in myocardial fibrosis: from basic science to clinical aspects. *Am J Physiol Heart Circ Physiol* 299: H1–H9, 2010.
99. **Lucattelli M, Cavarra E, de Santi MM, Tetley TD, Martorana PA, Lungarella G.** Collagen phagocytosis by lung alveolar macrophages in animal models of emphysema. *Eur Respir J* 22: 728–734, 2003.
100. **Luckhardt TR, Muller-Quernheim J, Thannickal VJ.** Update in diffuse parenchymal lung disease 2011. *Am J Respir Crit Care Med* 186: 24–29, 2012.
101. **Madri JA, Furthmayr H.** Collagen polymorphism in the lung. An immunochemical study of pulmonary fibrosis. *Hum Pathol* 11: 353–366, 1980.
102. **Madsen DH, Engelholm LH, Ingvarsen S, Hillig T, Wagenaar-Miller RA, Kjoller L, Gardsvoll H, Hoyer-Hansen G, Holmbeck K, Bugge TH, Behrendt N.** Extracellular collagenases and the endocytic receptor, urokinase plasminogen activator receptor-associated protein/Endo180, cooperate in fibroblast-mediated collagen degradation. *J Biol Chem* 282: 27037–27045, 2007.
103. **Madsen DH, Ingvarsen S, Jurgensen HJ, Melander MC, Kjoller L, Moyer A, Honore C, Madsen CA, Garred P, Burgdorf S, Bugge TH, Behrendt N, Engelholm LH.** The non-phagocytic route of collagen uptake: a distinct degradation pathway. *J Biol Chem* 286: 26996–27010, 2011.
104. **Maki JM.** Lysyl oxidases in mammalian development and certain pathological conditions. *Histol Histopathol* 24: 651–660, 2009.
105. **Martinez-Pomares L, Wienke D, Stillion R, McKenzie EJ, Arnold JN, Harris J, McGreal E, Sim RB, Isacke CM, Gordon S.** Carbohydrate-independent recognition of collagens by the macrophage mannose receptor. *Eur J Immunol* 36: 1074–1082, 2006.
106. **Matsuoka H, Sisson TH, Nishiuma T, Simon RH.** Plasminogen-mediated activation and release of hepatocyte growth factor from extracellular matrix. *Am J Respir Cell Mol Biol* 35: 705–713, 2006.
107. **Matsusaka H, Ide T, Matsushima S, Ikeuchi M, Kubota T, Sunagawa K, Kinugawa S, Tsutsui H.** Targeted deletion of matrix metalloproteinase 2 ameliorates myocardial remodeling in mice with chronic pressure overload. *Hypertension* 47: 711–717, 2006.
108. **Mays PK, McAnulty RJ, Laurent GJ.** Age-related changes in lung collagen metabolism. A role for degradation in regulating lung collagen production. *Am Rev Respir Dis* 140: 410–416, 1989.
109. **McAnulty RJ, Laurent GJ.** Collagen synthesis and degradation in vivo. Evidence for rapid rates of collagen turnover with extensive degradation of newly synthesized collagen in tissues of the adult rat. *Coll Relat Res* 7: 93–104, 1987.
110. **McCulloch CA.** Drug-induced fibrosis: interference with the intracellular collagen degradation pathway. *Curr Opin Drug Discov Devel* 7: 720–724, 2004.
111. **McCulloch CA, Knowles GC.** Deficiencies in collagen phagocytosis by human fibroblasts in vitro: a mechanism for fibrosis? *J Cell Physiol* 155: 461–471, 1993.
112. **McGaw WT, Porter H.** Cyclosporine-induced gingival overgrowth: an ultrastructural stereologic study. *Oral Surg Oral Med Oral Pathol* 65: 186–190, 1988.
113. **McGaw WT, Ten Cate AR.** A role for collagen phagocytosis by fibroblasts in scar remodeling: an ultrastructural stereologic study. *J Invest Dermatol* 81: 375–378, 1983.
114. **Meduri GU, Belenchia JM, Estes RJ, Wunderink RG, el Torky M, Leeper KV Jr.** Fibroproliferative phase of ARDS. Clinical findings and effects of corticosteroids. *Chest* 100: 943–952, 1991.
115. **Messaritou G, East L, Roghi C, Isacke CM, Yarwood H.** Membrane type-1 matrix metalloproteinase activity is regulated by the endocytic collagen receptor Endo180. *J Cell Sci* 122: 4042–4048, 2009.
116. **Mignatti P, Rifkin DB.** Biology and biochemistry of proteinases in tumor invasion. *Physiol Rev* 73: 161–195, 1993.
117. **Monea S, Lehti K, Keski-Oja J, Mignatti P.** Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism. *J Cell Physiol* 192: 160–170, 2002.
118. **Montano M, Ramos C, Gonzalez G, Vadillo F, Pardo A, Selman M.** Lung collagenase inhibitors and spontaneous and latent collagenase activity in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. *Chest* 96: 1115–1119, 1989.
119. **Montfort I, Perez-Tamayo R.** Collagenase in experimental carbon tetrachloride cirrhosis of the liver. *Am J Pathol* 92: 411–420, 1978.
120. **Mubarak KK, Montes-Worboys A, Regev D, Nasreen N, Mohammed KA, Faruqi I, Hensel E, Baz MA, Akindipe OA, Fernandez-Bussy S, Nathan SD, Antony VB.** Parenchymal trafficking of pleural mesothelial cells in idiopathic pulmonary fibrosis. *Eur Respir J* 39: 133–140, 2012.
121. **Muro AF, Moretti FA, Moore BB, Yan M, Atrasz RG, Wilke CA, Flaherty KR, Martinez FJ, Tsui JL, Sheppard D, Baralle FE, Toews GB, White ES.** An essential role for fibronectin extra type III domain A in pulmonary fibrosis. *Am J Respir Crit Care Med* 177: 638–645, 2008.
122. **Murphy G, Ward R, Gavrilovic J, Atkinson S.** Physiological mechanisms for metalloproteinase activation. *Matrix* 1 *Suppl*: 224–230, 1992.
123. **Nasreen N, Mohammed KA, Mubarak KK, Baz MA, Akindipe OA, Fernandez-Bussy S, Antony VB.** Pleural mesothelial cell transformation into myofibroblasts and haptotactic migration in response to TGF-β1 in vitro. *Am J Physiol Lung Cell Mol Physiol* 297: L115–L124, 2009.
124. **Nerenberg PS, Salsas-Escat R, Stultz CM.** Do collagenases unwind triple-helical collagen before peptide bond hydrolysis? Reinterpreting experimental observations with mathematical models. *Proteins* 70: 1154–1161, 2008.
125. **Nimni ME.** Collagen: structure, function, and metabolism in normal and fibrotic tissues. *Semin Arthritis Rheum* 13: 1–86, 1983.
126. **Nimni ME, De Guia E, Bavetta LA.** Synthesis and turnover of collagen precursors in rabbit skin. *Biochem J* 102: 143–147, 1967.
127. **Oda T, Jung YO, Kim HS, Cai X, Lopez-Guisa JM, Ikeda Y, Eddy AA.** PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction. *Kidney Int* 60: 587–596, 2001.
128. **Okazaki I, Maruyama K.** Collagenase activity in experimental hepatic fibrosis. *Nature* 252: 49–50, 1974.
129. **Okuma T, Terasaki Y, Kaikita K, Kobayashi H, Kuziel WA, Kawasuji M, Takeya M.** C-C chemokine receptor 2 (CCR2) deficiency improves bleomycin-induced pulmonary fibrosis by attenuation of both macrophage infiltration and production of macrophage-derived matrix metalloproteinases. *J Pathol* 204: 594–604, 2004.
130. **Orgel JP, Irving TC, Miller A, Wess TJ.** Microfibrillar structure of type I collagen in situ. *Proc Natl Acad Sci USA* 103: 9001–9005, 2006.
131. **Orgel JP, San Antonio JD, Antipova O.** Molecular and structural mapping of collagen fibril interactions. *Connect Tissue Res* 52: 2–17, 2011.
132. **Pardo A, Selman M.** Matrix metalloproteases in aberrant fibrotic tissue remodeling. *Proc Am Thorac Soc* 3: 383–388, 2006.
133. **Perez-Ramos J, de Lourdes Segura-Valdez M, Vanda B, Selman M, Pardo A.** Matrix metalloproteinases 2, 9, and 13, and tissue inhibitors of metalloproteinases 1 and 2 in experimental lung silicosis. *Am J Respir Crit Care Med* 160: 1274–1282, 1999.
134. **Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM.** Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 114: 438–446, 2004.

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135. **Pierce JA, Resnick H, Henry PH.** Collagen and elastin metabolism in the lungs, skin, and bones of adult rats. *J Lab Clin Med* 69: 485–493, 1967.
136. **Radbill BD, Gupta R, Ramirez MC, DiFeo A, Martignetti JA, Alvarez CE, Friedman SL, Narla G, Vrabie R, Bowles R, Saiman Y, Bansal MB.** Loss of matrix metalloproteinase-2 amplifies murine toxin-induced liver fibrosis by upregulating collagen I expression. *Dig Dis Sci* 56: 406–416, 2011.
137. **Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ.** Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 436: 123–127, 2005.
138. **Raghu G, Brown KK, Costabel U, Cottin V, du Bois RM, Lasky JA, Thomeer M, Utz JP, Khandker RK, McDermott L, Fatenejad S.** Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. *Am J Respir Crit Care Med* 178: 948–955, 2008.
139. **Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ.** An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 183: 788–824, 2011.
140. **Raghu G, Striker LJ, Hudson LD, Striker GE.** Extracellular matrix in normal and fibrotic human lungs. *Am Rev Respir Dis* 131: 281–289, 1985.
141. **Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP.** Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. *J Biol Chem* 274: 13066–13076, 1999.
142. **Reiser KM, Tryka AF, Lindenschmidt RC, Last JA, Witschi HR.** Changes in collagen cross-linking in bleomycin-induced pulmonary fibrosis. *J Biochem Toxicol* 1: 83–91, 1986.
143. **Riley DJ, Berg RA, Soltys RA, Kerr JS, Guss HN, Curran SF, Laskin DL.** Neutrophil response following intratracheal instillation of collagen peptides into rat lungs. *Exp Lung Res* 14: 549–563, 1988.
144. **Romijn RA, Bouma B, Wuyster W, Gros P, Kroon J, Sixma JJ, Huizinga EG.** Identification of the collagen-binding site of the von Willebrand factor A3-domain. *J Biol Chem* 276: 9985–9991, 2001.
145. **Rozin GF, Gomes MM, Parra ER, Kairalla RA, de Carvalho CR, Capelozzi VL.** Collagen and elastic system in the remodelling process of major types of idiopathic interstitial pneumonias (IIP). *Histopathology* 46: 413–421, 2005.
146. **San Antonio JD, Lander AD, Karnovsky MJ, Slayter HS.** Mapping the heparin-binding sites on type I collagen monomers and fibrils. *J Cell Biol* 125: 1179–1188, 1994.
147. **Schneider F, Sukhova GK, Aikawa M, Canner J, Gerdes N, Tang SM, Shi GP, Apte SS, Libby P.** Matrix-metalloproteinase-14 deficiency in bone-marrow-derived cells promotes collagen accumulation in mouse atherosclerotic plaques. *Circulation* 117: 931–939, 2008.
148. **Schrier DJ, Phan SH, Ward PA.** Cellular sensitivity to collagen in bleomycin-treated rats. *J Immunol* 129: 2156–2159, 1982.
149. **Schuppan D.** Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Semin Liver Dis* 10: 1–10, 1990.
150. **Selman M, Chapela R, Montano M, Soto H, Diaz de Leon L.** Changes of collagen content in fibrotic lung disease. *Arch Invest Med (Mex)* 13: 93–100 passim, 1982.
151. **Selman M, Montano M, Ramos C, Chapela R.** Concentration, biosynthesis and degradation of collagen in idiopathic pulmonary fibrosis. *Thorax* 41: 355–359, 1986.
152. **Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, Pardo A.** TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *Am J Physiol Lung Cell Mol Physiol* 279: L562–L574, 2000.
153. **Sen AI, Shiomi T, Okada Y, D'Armiento JM.** Deficiency of matrix metalloproteinase-13 increases inflammation after acute lung injury. *Exp Lung Res* 36: 615–624, 2010.
154. **Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, Gabbiani G.** The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol* 142: 873–881, 1998.
155. **Seyer JM, Hutcheson ET, Kang AH.** Collagen polymorphism in idiopathic chronic pulmonary fibrosis. *J Clin Invest* 57: 1498–1507, 1976.
156. **Shi F, Harman J, Fujiwara K, Sottile J.** Collagen I matrix turnover is regulated by fibronectin polymerization. *Am J Physiol Cell Physiol* 298: C1265–C1275, 2010.
157. **Shieh DH, Chiang LC, Lee CH, Yang YH, Shieh TY.** Effects of arecoline, safrole, and nicotine on collagen phagocytosis by human buccal mucosal fibroblasts as a possible mechanism for oral submucous fibrosis in Taiwan. *J Oral Pathol Med* 33: 581–587, 2004.
158. **Siwik DA, Chang DL, Colucci WS.** Interleukin-1beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circ Res* 86: 1259–1265, 2000.
159. **Smith C, Estavillo D, Emsley J, Bankston LA, Liddington RC, Cruz MA.** Mapping the collagen-binding site in the I domain of the glycoprotein Ia/IIa (integrin $\alpha_2\beta_1$). *J Biol Chem* 275: 4205–4209, 2000.
160. **Snider GL.** Collagen concentration and rates of synthesis in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 124: 341–342, 1981.
161. **Song F, Wisithphrom K, Zhou J, Windsor LJ.** Matrix metalloproteinase dependent and independent collagen degradation. *Front Biosci* 11: 3100–3120, 2006.
162. **Srivastava M, Steinwede K, Kiviranta R, Morko J, Hoymann HG, Langer F, Buhling F, Welte T, Maus UA.** Overexpression of cathepsin K in mice decreases collagen deposition and lung resistance in response to bleomycin-induced pulmonary fibrosis. *Respir Res* 9: 54, 2008.
163. **Starcher BC, Kuhn C, Overton JE.** Increased elastin and collagen content in the lungs of hamsters receiving an intratracheal injection of bleomycin. *Am Rev Respir Dis* 117: 299–305, 1978.
164. **Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, Thompson BT, Ancukiewicz M.** Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. *N Engl J Med* 354: 1671–1684, 2006.
165. **Strieter RM, Mehrad B.** New mechanisms of pulmonary fibrosis. *Chest* 136: 1364–1370, 2009.
166. **Stultz CM.** Localized unfolding of collagen explains collagenase cleavage near imino-poor sites. *J Mol Biol* 319: 997–1003, 2002.
167. **Suga M, Iyonaga K, Okamoto T, Gushima Y, Miyakawa H, Akaike T, Ando M.** Characteristic elevation of matrix metalloproteinase activity in idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 162: 1949–1956, 2000.
168. **Sweeney SM, Orgel JP, Fertala A, McAuliffe JD, Turner KR, Di Lullo GA, Chen S, Antipova O, Perumal S, Ala-Kokko L, Forlino A, Cabral WA, Barnes AM, Marini JC, San Antonio JD.** Candidate cell and matrix interaction domains on the collagen fibril, the predominant protein of vertebrates. *J Biol Chem* 283: 21187–21197, 2008.
169. **Takamiya Y, Fukami K, Yamagishi SI, Kaida Y, Nakayama Y, Obara N, Iwatani R, Ando R, Koike K, Matsui T, Nishino Y, Ueda S, Cooper ME, Okuda S.** Experimental diabetic nephropathy is accelerated in matrix metalloproteinase-2 knockout mice. *Nephrol Dial Transplant* 28: 55–62, 2013.
170. **Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S.** Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol* 42: 561–568, 2006.
171. **Trentham DE, Townes AS, Kang AH, David JR.** Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats. *J Clin Invest* 61: 89–96, 1978.
172. **Tsai CC, Ma RH, Shieh TY.** Deficiency in collagen and fibronectin phagocytosis by human buccal mucosa fibroblasts in vitro as a possible mechanism for oral submucous fibrosis. *J Oral Pathol Med* 28: 59–63, 1999.
173. **Uchinami H, Seki E, Brenner DA, D'Armiento J.** Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. *Hepatology* 44: 420–429, 2006.
174. **Ueno M, Maeno T, Nomura M, Aoyagi-Ikeda K, Matsui H, Hara K, Tanaka T, Iso T, Suga T, Kurabayashi M.** Hypoxia-inducible factor-1α mediates TGF-β-induced PAI-1 production in alveolar macrophages in pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 300: L740–L752, 2011.
175. **van der Zee E, Everts V, Hoeben K, Beertsen W.** Cytokines modulate phagocytosis and intracellular digestion of collagen fibrils by fibroblasts in rabbit periosteal explants. Inverse effects on procollagenase production and collagen phagocytosis. *J Cell Sci* 108: 3307–3315, 1995.

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176. **van Zuijlen PP, Ruurda JJ, van Veen HA, van Marle J, van Trier AJ, Groenevelt F, Kreis RW, Middelkoop E.** Collagen morphology in human skin and scar tissue: no adaptations in response to mechanical loading at joints. *Burns* 29: 423–431, 2003.
177. **Verhaegen PD, van Zuijlen PP, Pennings NM, van Marle J, Niessen FB, van der Horst CM, Middelkoop E.** Differences in collagen architecture between keloid, hypertrophic scar, normotrophic scar, and normal skin: an objective histopathological analysis. *Wound Repair Regen* 17: 649–656, 2009.
178. **Visscher DW, Myers JL.** Histologic spectrum of idiopathic interstitial pneumonias. *Proc Am Thorac Soc* 3: 322–329, 2006.
179. **von Delwig A, Hilkens CM, Altmann DM, Holmdahl R, Isaacs JD, Harding CV, Robertson H, McKie N, Robinson JH.** Inhibition of macropinocytosis blocks antigen presentation of type II collagen in vitro and in vivo in HLA-DR1 transgenic mice. *Arthritis Res Ther* 8: R93, 2006.
180. **Wang H, Zhang Y, Heuckeroth RO.** PAI-1 deficiency reduces liver fibrosis after bile duct ligation in mice through activation of tPA. *FEBS Lett* 581: 3098–3104, 2007.
181. **Wang X, Zhou Y, Tan R, Xiong M, He W, Fang L, Wen P, Jiang L, Yang J.** Mice lacking the matrix metalloproteinase-9 gene reduce renal interstitial fibrosis in obstructive nephropathy. *Am J Physiol Renal Physiol* 299: F973–F982, 2010.
182. **Weathington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, Folkerts G, Nijkamp FP, Blalock JE.** A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. *Nat Med* 12: 317–323, 2006.
183. **Wess TJ, Hammersley AP, Wess L, Miller A.** A consensus model for molecular packing of type I collagen. *J Struct Biol* 122: 92–100, 1998.
184. **Westergren-Thorsson G, Sime P, Jordana M, Gauldie J, Sarnstrand B, Malmstrom A.** Lung fibroblast clones from normal and fibrotic subjects differ in hyaluronan and decorin production and rate of proliferation. *Int J Biochem Cell Biol* 36: 1573–1584, 2004.
185. **Yamashita CM, Dolgonos L, Zemans RL, Young SK, Robertson J, Briones N, Suzuki T, Campbell MN, Gauldie J, Radisky DC, Riches DW, Yu G, Kaminski N, McCulloch CA, Downey GP.** Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. *Am J Pathol* 179: 1733–1745, 2011.
186. **Zhang D, Leung N, Weber E, Saftig P, Bromme D.** The effect of cathepsin K deficiency on airway development and TGF-beta1 degradation. *Respir Res* 12: 72, 2011.
187. **Zhang G, Ezura Y, Chervoneva I, Robinson PS, Beason DP, Carine ET, Soslowsky LJ, Iozzo RV, Birk DE.** Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. *J Cell Biochem* 98: 1436–1449, 2006.
188. **Zhang K, Rekhter MD, Gordon D, Phan SH.** Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. *Am J Pathol* 145: 114–125, 1994.
189. **Zhao BQ, Ikeda Y, Ihara H, Urano T, Fan W, Mikawa S, Suzuki Y, Kondo K, Sato K, Nagai N, Umemura K.** Essential role of endogenous tissue plasminogen activator through matrix metalloproteinase 9 induction and expression on heparin-produced cerebral hemorrhage after cerebral ischemia in mice. *Blood* 103: 2610–2616, 2004.
190. **Zhou Y, Huang X, Hecker L, Kurundkar D, Kurundkar A, Liu H, Jin TH, Desai L, Bernard K, Thannickal VJ.** Inhibition of mechanosensitive signaling in myofibroblasts ameliorates experimental pulmonary fibrosis. *J Clin Invest* 123: 1096–1108, 2013.
191. **Zhuge Y, Xu J.** Rac1 mediates type I collagen-dependent MMP-2 activation. Role in cell invasion across collagen barrier. *J Biol Chem* 276: 16248–16256, 2001.
192. **Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, Sheppard D, Pardo A, Selman M, Heller RA.** Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 99: 6292–6297, 2002.

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